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Cell Adhesion Inhibitor

[細胞接着抑制剤]

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(54) [Title of the Invention] Cell Adhesion Inhibitor

(57) [Abstract]

[Problem] To provide a cell adhesion inhibitor and a cancer metastasis inhibitor

[Solution] This inhibitor comprises one or more plants selected from the group consisting of Chrysanthemum spp., Terminalia chebula Retz., Trapa japonica FLEROV., Geum japonicum THUNB., Punica granatum L., Arctostaphylos uva-ursi SPRENGEL, Achillea millefolium, Rosa spp., Cuminum cynimum and Eugenia aromatica/Syzygium aromaticum or one or more plants selected from the group consisting of Solidago virga-aurea, Polygalae radix, Gymnema sylvestra (Retz.) Schult., Symphytum officinale L., Dianthus superbus L./D. chinensis L., Platycodon grandiflorum A. DC., Calendula officinalis L., Capsicum annuum L., Gynostemma pentaphyllum (Thunb.) Makino, Bupleurum falcatum L., Anemarrhena asphodeloides Bunge, Humulus lupulus L. and Lonicera japonica Thunb. or their extracts as active ingredients.

[Claims]

[Claim 1] The cell adhesion inhibitor which makes an active ingredient of one or plants chosen from the group which consists of a chrysanthemum, Terminalia chebula Retzus, a water caltrop, Japanese radish grass, a pomegranate real hide, a bearberry leaf, YARO, Lowe's, cumin, a clove, a Solidago japonica, a polygala root, Gymnema, a comfrey, Herba Dianthi Superbi, a platycodi radix, a pot marigold, red pepper, a city yaw tongue, Phycho, an anemarrhena

rhizome, hop, and Lonicera japonica, or their extracts.

[Claim 2] The cancer metastasis inhibitor which makes an active ingredient of one or more plants chosen from the group which consists of a chrysanthemum, Terminalia chebula Retzus, a water caltrop, Japanese radish grass, a pomegranate real hide, a bearberry leaf, YARO, Lowe's, cumin, a clove, a Solidago japonica, a polygala root, Gymnema, a comfrey, Herba Dianthi Superbi, a platycodi radix, a pot marigold, red pepper, a city yaw tongue, Phycho, an anemarrhena rhizome, hop, and Lonicera japonica, or its extract.

[Claim 3] A food product composition which is used for cell adhesion inhibition which includes plants of one or more than 2 types that are selected from the group of chrysanthemum, Terminalia chebula Retzus, a water caltrop, Japanese radish grass, a pomegranate real hide, a bearberry leaf, YARO, Lowe's, cumin, a clove, a Solidago japonica, a polygala root, Gymnema, a comfrey, Herba Dianthi Superbi, a platycodi radix, a pot marigold, red pepper, a city yaw tongue, Phycho an anemarrhena rhizome, hop, and Lonicera japonica or their extracts and food product ingredients.

[Claim 4] A food product composition which is used for cancer metastasis inhibition which includes plants of one

or more than 2 types that are selected from the group of chrysanthemum, Terminalia chebula Retzus, a water caltrop, Japanese radish grass, a pomegranate real hide, a bearberry leaf, YARO, Lowe's, cumin, a clove, a Solidago japonica, a polygala root, Gymnema, a comfrey, Herba Dianthi Superbi, a platycodi radix, a pot marigold, red pepper, a city yaw tongue, Phycho an anemarrhena rhizome, hop, and Lonicera japonica or their extracts and food product ingredients.

[Detailed Explanation of the Invention]

[0001]

[Technical Field of the Invention]This invention relates to a food product composition which has a cell adhesion inhibitor and cancer metastasis inhibitor with plant or their extracts as active ingredients and which are used for cell adhesion prevention or cancer metastasis control.

[0002]

[Description of the Prior Art] Recently, the importance of cell adhesion molecules in inflammatory reactions is becoming gradually clear through research on cell adhesion molecules. As a reaction to inflammation within the body, there is migration to the vascular endothelial cells of leukocytes and from this, the leukocytes, which flows

through the blood, rolls gently on top of a vascular endothelial cell, stops, and soon strongly adheres to the cell and the process, by which migration is carried to the outside of the blood vessel, is known. It is thought that inflammation originates in the adhesion interaction between the above-mentioned leukocyte and a vascular endothelial cell, and it has also become clear that the fixed adhesion molecule plays an important role as a factor in adhesion interaction. Moreover, adhesion molecules exist within leukocytes and activated vascular endothelial cells (blood vessel of inflamed sites), and it is known that the interaction of these adhesion molecules is the initial secretion stage related to the infiltration of the leukocyte to the inflamed area ("Sugar Chain Hybrid", Kyoritsu Publishing 1995, Experimental Medicine Bioscience, "World of Cell Adhesion Molecules", Kyoritsu Publishing, 1995).

[0003] If explained concretely, it is thought that for the adhesion of the leukocytes and the vascular endothelial cells, there are four stages to the process, (1) rolling of the leukocyte which gives selectin, (2) activation of the leukocyte, (3) strong adhesion through the participation of LFA-1, Mac-1 integrin and Ig super family ICAM-1, ICAM-2,

and VCAM, and (4) vascular wall passage. Consequently, if it is possible to control the process of any of these processes, it is thought inflammation can be affected.

[0004]

[0004] In particular, for the (1) stage, selectin within the blood initially adheres to the leukocyte, slowing the flow of the leukocyte, and together with causing the leukocytes to roll, because there is a process in which there is transmission of site information of the inflammation site or the establishment of a mobilized cell, if it is possible to control the adhesion of (1), that is, if there can be controlled the adhesion of the adhesion molecules (E-selectin) among the leukocytes within the blood vessel or prevention of the phemonenon of cell adhesion molecules (E-selectin), the rolling of the leukocytes can be matched,and in addition, afterwards control occurs for the stages (2)~ (4) , and it is thought that there is an unusually high probability that the cell adhesion inhibitor would be able to counter the inflammation (Glycobiology Series 6 Glycobiology Kodansha in 1993). Immunology highlights Coordinator of Adhesion Molecules Immunoreactions, Chugai Medicine, 1995).

[0005] On the other hand, although cancer continues to be a sickness that can be subdued or cured by treatment and diagnostics, there is currently no effective treatment for cancer metastasis. Recently, from progress made in molecular biology, the metastasis process of cancer cells has become clear, and the mutual interaction among cells through cell adhesion molecules for hematogenic metastasis has become clear. ("Molecular Medicine for New Clinician Series", Molecular Medicine of Cancer Metastasis (Yodosha, 1996)). In cancer metastasis, there are the 3 principal roots of disseminated metastasis, lymph node metastasis, and hematogenic metastasis, but, even among these, hematogenic metastasis is seen as important because metastasis occurs in remote organs. Cancer cells occur by attachment to remote organs through the blood flow of cancer cells from hematogenic metastasis. For the adhesion towards remote organ cells of these cancer cells, it has been reported that cancer adhesion molecules first participate as E-selectin. That is, for the selectin, there is a strong ability to adhere by trapping the cells (ligand sugar chain of a cancer cell) that flow at high speed through the blood, and selectin is thought to participate in the initial stage of the adhesion towards the blood vessel's endodermis. Consequently, from the finding of

substances that can prevent directly (adhesion prevention) or indirectly (E-selectin manifestation) the adhesion the selectin and the ligand sugar chain, it is thought that the desired control of cancer metastasis will be obtained (Progress of Medicine VOL.179 No.2 1996.10.12129-134).

[0006] Moreover, although strong drugs with adverse reactions are used in the treatment of cancer, and it is possible to exterminate for a short period the cancer, it is not possible to maintain the patient's quality of life (quality of life). Unlike these drugs, metastasis is prevented, and the growth of micrometastasis is completely suppressed, and there is provided a complement to resistance relative to the host cancer, making these drugs a desirable treatment agent for maintaining for a longtime the patient's QOL. These metastasis control substances are used as complementary treatment agents for controlling the metastasis before and after surgery or radiation treatment, and it is expected they will also find use as a combination therapy agent.

[0007]

[Problem(s) to be Solved by the Invention] Therefore, the purpose of this invention is to control the mechanism of adhesion to the vascular endothelial cell of inflammation

related cells, such as a leukocyte and a cancer cell in therapy or prevention. It is in specifically offered as an effective inhibitor and effective cancer metastasis inhibitor, and as it has been found that there are hardly any adverse reactions from herbal medicine oriented plants, they are presented as drug products, non-drug products, and food products for possible long term use.

[0008] [Means for Solving the Problem] The inventors investigated cell adhesion control use for various plants or their extracts, and the plants that are indicated below, unexpectedly, have superior cell adhesion control use, and it was found that they were extremely useful as cancer metastasis control inhibitors, completely the invention.

[0009] That is, this invention offers cell adhesion inhibitors and cancer metastasis inhibitors, acting as active ingredients, from the extracts of one or more than 2 kinds of plants which are selected from a chrysanthemum, Terminalia chebula Retzus, a water caltrop, Japanese radish grass, a pomegranate real hide, a bearberry leaf, YARO, Lowe's, cumin, and a clove.

[0010] Furthermore, this invention offers the cell adhesion inhibitor and cancer transition inhibitor which make an active ingredient of one kind or two kinds or more of

plants chosen from the group which consists of a Solidago japonica, a polygala root, Gymnema, a comfrey, Herba Dianthi Superbi, a platycodi radix, a pot marigold, red pepper, a city yaw tongue, Phycho, an anemarrhena rhizome, hop, and Lonicera japonica, or its extract.

[0011] The plants used by this invention are a chrysanthemum (Chrysanthemum spp.), Terminalia chebula Retzus (Terminalia chebula Retz.), a water caltrop (Trapa japonica FLEROV.), Japanese radish grass (Geum japonicum THUNB.), a pomegranate real hide (Punica granatum L.), a bearberry leaf (Arctostaphylos uva-ursi SPRENGEL), YARO (Achillea millefolium), Lowe's (Rosa spp.), cumin (Cuminum cynimum), and a clove (Eugenia aromatica/Syzygium aromaticum).

[0012] Other plants used by this invention include a Solidago japonica (Solidago virga-aurea), a polygala root (Polygalae radix), Gymnema Silvester (Gymnema sylvestre(Retz.) Schult.), a comfrey (powder of the rhizome of Symphytum officinale (Symphytum officinale L.)), Herba Dianthi Superbi (a pink/Dianthus chinensis (Dianthus superbus L./D.chinensis L.)), a platycodi radix (Platycodon

grandiflorum A.DC.), a pot marigold (common marigold (Calendula officinalis L.)), red pepper (Capsicum annuum L.), a yaw tongue (Gynostemma pentaphyllum (Gynostemma pentaphyllum(Thunb.) Makino)), Phycho (Bupleurum (Bupleurum falcatum L.)), an anemarrhena rhizome (amenarrhena (Anemarrhena asphodeloides Bunge) --), especially chosen from a rhizome, hop (SEIYOUKARAHANASOU) (Humulus lupulus L.), and the group that consists of an ear and Lonicera japonica (Japanese honeysuckle (Lonicera japonica Thunb.), especially the leaf).

[0013] A chrysanthemum that is the dried capitulum of chrysanthemums, such as a white chrysanthemum of Compositae (Compositae), [Unknown], [Unknown], and [Unknown], and in Japan the chrysanthemum is considered edible.

[0014] Terminalia chebula Retzus is a kind of herbal medicine which has dried the mature fruit of the Terminalia chebula (Terminalia chebula Retz.) of the plant Combretaceae.

[0015] A water caltrop is a kind of herbal medicine which has dried the fruit of Hishi of the (Trapaceae) pant of a water chestnut, and is made edible.

[0016] Japanese radish grass is from drying and then mincing the entire plant of Geum japonicum of the plant Rosaceae.

[0017] A pomegranate's skin is a kind of herbal medicine which is produced by drying the pericarp of the pomegranate of the Punicaceae plant.

[0018] A bearberry leaf is an herbal medicine which has dried the leaf of the Arctostaphylos uva-ursi of Ericaceae.

[0019] YARO is the Japanese name for a western [Unknown], and is the plant of Compositae, drunk as tea in Europe.

[0020] The rose is a kind of herb which results from drying the flower of the rose of Rosaceae, and is made edible.

[0021] Cumin is the plant of Umbelliferae and its seeds are mainly used mainly as a spice.

[0022] The clove is from drying the bud of the Japanese-named caryophylli flos of Myrtaceae (Myrtaceae), and is a kind of a spice.

[0023] A Solidago japonica is the entire plant of the Solidago japonica of Compositae (Compositae), and is drunk as tea.

[0024] A polygala root is the plant of Polygalaceae and is the root of Polygala tenuifolia.

[0025] Gymnema is the leaf of Gymnema Silvester of Asclepiadaceae (Asclepiadaceae).

[0026] A comfrey is the plant of a gromwell (Boraginaceae) and is the powder of the rhizome of Symphytum officinale (Symphytum officinale L.).

[0027] Herba Dianthi Superbi results from drying the KAWARA pink of Caryophyllaceae (Caryophyllaceae), and the entire plant is the flower of Dianthus chinensis.

[0028] A platycodi radix is the root of the platycodi radix of Campanulaceae (Campanulaceae).

[0029] A pot marigold is the Japanese name for a common marigold, and the flower is edible as is the plant of Compositae (Compositae).

[0030] Red pepper is the Japanese name for a red pepper, is the mature fruits of the plant of Solanaceae (Solanaceae), and is mainly used as a spice.

[0031] A city yaw tongue is the leaf of AMACHAZURU of Cucurbitaceae (Cucurbitaceae).

[0032] Phycho is Bupleurum of Umbelliferae (Umbelliferae), or the root.

[0033] An anemarrhena rhizome is the rhizome of the amenarrhena of Liliaceae (Liliaceae).

[0034] Hop is the Japanese name for SEIYOUKARAHANASOU, is the flower's ear and [Unknown] of plant hop of Moraceae (Moraceae), and is made edible.

[0035] Lonicera japonica is from drying the leaf of the Japanese honeysuckle of Caprifoliaceae (Caprifoliaceae).

[0036] In this invention, the entire vegetation of the plant such as a leaf, a petiole, a flower, fruits, a lateral root, a seed, etc. can be used. It is permissible to dry as it and use, perhaps grinding it and using it as an extract. Preferably, the extract of said plant is used.

[0037] There have been citations for extraction methods which extract one part of the plant or a powdery substance of the entire body from water or an organic solvent usually at 3 ~ 100°C. The organic solvent which is used for the extract can be, but is not limited to, alcohols, such as ketones, such as ester, such as halogenated hydrocarbon, such as hydrocarbons, such as the petroleum ether, a cyclohexane, toluene, and benzene, a carbon tetrachloride, dichloromethane, and chloroform, ether, and ethyl acetate, and an acetone, a butanol, propanol, ethanol, a methanol, a polyethylene glycol, propylene glycol, and a butylene glycol, and a pyridine. An extracting solvent may be used independently, or one may mix and use two or more kinds. Preferably, hydrous alcohol, such as hydrous ethanol and hydrous methanol, can be used. Moreover, the preferable method is to extract by using the dried powder of natural products with water 20 times the weight of the powder, 50% ethanol at 20 times the weight, or 50 times the weight in ethanol, and stir for 2~24 hours at from room temperature to 100°C.

[0038] Although the obtained extract may be used as is, according to requirements, it is also permissible to use

the product resulting from such processes as concentration, filtering, and freeze drying. In addition, the extract or the plant can be used independently as a combination of more than two kinds.

[0039] The above-mentioned plant extract obtained in this way is utilized outstandingly to control the cell adhesion which is represented between the leukocyte-vascular endothelial cells. In addition, the extract can be used as an outstanding cancer metastasis inhibitor. In addition, cytotoxicity is weak, and safety is high. Consequently, the drug product, the non-drug product, and the food product which uses the above-mentioned plant extract, based on its cell adhesion control, is used in the prevention and treatment of cancer metastasis.

[0040] The blending quantities of drug product, non-drug product and food product of the above-mentioned plants and their extracts are not especially limited, but, generally, are calculated by portion of the dried solids, and it is desirable to have the weight in the range of 0.001 ~ 100. wt. %; or more preferably, in the range of 0.01 ~ 50 wt. %. In addition, the first day's dose (and intake) is desirable if in the range of 0.1mg/kg ~ 1000mg/kg, with

administration divided in one times/day or several times/day.

[0041] Administration can be done by any of the pathways, orally, non-orally, and externally (ointment, emulsion, cream pharmaceuticals, or patches). In addition, the medicine can blended as a food additive in food products for specific health needs, JSD food products, special nutritive foods, supplements, and health foods. In addition, it is known that the safety of these plants and extracts is high.

[0042] As an working example, an arbitrary form may be taken if there is the expectation of a cell adhesion inhibitor effect or a cancer metastasis inhibit effect, and the following have been mentioned: solids (pharmaceutical preparation), such as a tablet, powder, a granule, a capsule, suppositories, and trochiscus, syrup, a milky lotion, a soft gelatin capsule, a cream, ointment, a fluid extract, suspension, a lotion, tincture, gel, a paste, a spray, injection, etc. are liquefied (pharmaceutical preparation), medicinal tea, a bath agent, and decoction.

[0043] It is desirable to blend the diluent and excipient used for preparation of the usual drugs, non-drugs, food products according to a working example for the pharmaceutical preparation of this invention. There is no special limitation for other cited additives.

[0044] As a food product ingredient which is included in this invention's food product composition, it is possible too add the extract of this invention with the expectation of a preventative and inhibiting effect, and it is equally possible to use the plant in salads, spices or teas. For foods which use additives, there is the possibility of using such additives in every type of food product, and the following are included in that category: drinks (tea, soft drink, etc.), confectionary, pans (cornflakes, cookies, a candy, jelly, etc.), noodles, boiled-fish-paste fats and oils (a sausage, boiled fish paste, etc.), seasonings (a dressing, sauce, etc.)

[0045] Drugs, non-drugs, food products, etc. of this invention can be manufactured using a conventional method.

[0046]

[Effect of the Invention] The plants used for this invention do not have adverse effects, and have outstanding

cell adhesion inhibition effects and cancer metastasis inhibition effect. Moreover, when they are used as drugs, non drugs, and food products, they are widely used for their effectiveness in the prevention and therapy of cancer.

[0047]

[Working Examples] Next, although an example is given and this invention is further explained in detail, this invention is not limited to these working examples.

[0048] Although an example shows an example of manufacturing the extract of said herbs and the extract's effectiveness is given for this invention as an example, these examples do not limit in any way this invention.

[0049] Manufacturing Example 1 (hot water extract)
The plant extract used in the following examples was obtained by the following method.

[0050]The dried chrysanthemum was ground up, and water, 20 times the amount was added to 2.0g of this powder, and extraction was accomplished by stirring for 2 hours at 95

°C. The precipitate was removed by centrifuging this extract (10,000 rpm, 15 min), and by freeze drying the supernatant, there was obtained 507mg of this extract (25% by weight of the desiccated solid). Even with other plants, the extract was obtained by the same operation.

[0051] Manufacturing Example 2 (methanol extract)

The dried chrysanthemum was ground up, and 50 times amount of methanol was added to 2.0g of this powder, the extraction accomplished by a Soxhlet extraction method employed for 2 hours at 100 °C. The precipitate was removed by centrifuging this extract liquid (10,000rpm, 15min), and after vacuum concentration of the supernatant, 241mg of the extract was obtained by freeze drying (12% of the weight of the desiccated solid). Even with other plants, the extract was obtained by the same operation.

[0052] Manufacturing Example 3 (50% ethanol extract)

The dry chrysanthemum was ground up, 50% ethanol of a 20 times amount was added to 2.0g of this powder, and the stirring of the extract was carried out at the room temperature for 24 hours. Centrifugal separation (10,000rpm, 15min) removed the precipitate, and 468mg (23 % of the weight of desiccated solid) of this extract was obtained

after vacuum concentration of the supernatant and after freeze drying. Even with other plants, the extract was obtained by the same operation.

[0053] Experiment 1

Leukocyte vascular endothelial cell adhesion inhibition test: investigated the substance which controls cells adhesion by countering the manifestation of adhesion molecules of vascular endothelial cells by the following experimental method.

[0054] Preparation of fluorescence label HL60 cell (human bone marrow tumor cell)

Using 2 x 10^7 HL60 cells and BCECG-AM 50μg, incubate for 30 minutes at 37°C, and fluorescence labeled. After washing with the new culture liquid, the cell concentration was prepared using the culture liquid so that there resulted in a concentration of 1 x 10^6 cells/ml.

[0055] Cell Adhesion Inhibition Experiment

To human vascular endothelial cells (HUVEC) which became confluent on 96 well flat plates (flat bottom plate), there was added subject extract in a final concentration

(desiccated solid form) (below, same) of 0.1mg/ml. After 18 hours, there was added 50μl/well of the human IL-1β (Genzyme product) so that the final concentration became 10 units/ml, and this solution was cultured for 4 hours at 37°C. After culture liquid removal, washing was done twice using the new culture liquid, and following previous usual methods, there was added100μl/well of the human bone marrow tumor cells (HL60) 1 x 10^6 cells/ml that were fluorescently labeled, and then reacted by a CO_2 incubator at 37°C. After 15 minutes, there was removal of the non-adhered cells, and every 100μl/well of new culture solution and 0.1% SDS solution was added, and the adhered cell was made soluble. There was a measurement of the fluorescent intensity after dissolving using the fluorescence plate reader (Ex490nm, Em530nm). In addition, instead of experimental materials, distilled water was used as a control, and there were blanks which did not have experimental materials and IL-1β and there was a request for the adhesion inhibition rate according to the equation. These results, as shown in Table 1, indicated that the extract substance had been determined to have the effect of inhibiting cell adhesion. In addition, in Table 2 there is shown the effect of the extracted substance when the 3 types of extraction methods were used (methods of manufacturing examples 1~3). When using any of

the extracts, cell adhesion inhibition effects were recognized.

[0056]

[Equation 1] Cell adhesion inhibition rate (%) = [1-{(experimental F value -blank F value)/ (contract F value - blank F value)}] x 100 [In the equation, the F value indicates the fluorescent intensity]

[0057]

[Table 1]

Plant Extracts (manufacturing example 3)	Leukocyte cell adhesion inhibition rate
Chrysanthemum	38
Terminalia chebula Retzus	88
Water caltrop	88
Japanese radish grass	52
Pomegrante actual skin	48

Bearberry leaf	99
YARO	29
Rose	41
Cumin	54
Clove	18

[0058]

[Table 2]

Leukocyte cell adhesion inhibition rate	
Chrysanthemum	
Manufacturing Example 1	27
Manufacturing Example 2	31
Manufacturing Example 3	38
Terminalia chebula Retzus	
Manufacturing Example 1	80
Manufacturing Example 2	95
Manufacturing Example 3	88

Experiment 2 Adhesion molecule manifestation inhibition experiment on vascular endothelial cells

For the substance that was seen as effective in Experiment 1 (manufacturing example 3), there were investigations as

to whether there was any manifestation of adhesion molecules relating to cell adhesion.

[0059]In 96 wells on a flat bottom plate (flat bottom plate), human vascular endothelial cells (HUVEC) were cultivated at 37 °C by a CO2 incubator until they became confluent. After washing the wells with culture liquid, experimental extract substance was added so that the final concentration became 0.1mg/ml, and then they were cultured. After 18 hours, human IL-1β (Genzyme product), 50μl/well was added so that the final concentration became 10 units/ml, and activation was done by incubation, and conduction manifestation of the adhesion molecules was implemented. After activation, the wells were washed with washing fluid (0.5% BSA-PBS+). 50μl of biotinized antibody solution (E-selectin; BBA8, ICAM-1;BBA9 (R&D Systems, product)) that was diluted by washing liquid 1000 and 2000 times was added, and the solution was allowed to remain standing at room temperature for one hour. The wells were then washed with washing liquid. There was the addition of 50μl of peroxidase labeled streptoavidin (Boehringer Mannheim product) solution that was diluted 2000 times by the washing liquid, and then the solution was allowed to

remain standing for 1 hour at room temperature. After the wells were washed with the washing liquid, 100μl of an acetic-acid buffer-solution (50mM sodium acetate buffer solution pH 5.2) and 100μl of a substrate solution (a 4mM o-phenylenediamine, 0.05% H 2O2/acetic-acid buffer solution) were added to the wells, and an enzyme reaction was performed at a room temperature for 5 minutes. The reaction was stopped by adding 50 μl of stopping solution (0.8M sulfuric acid. Measurements were made of the measured wavelength 492nm with the microplate reader, and measurements made of the absorbance at a control wavelength of 630nm. In addition, in place of the experimental material, purified water was used as a control, and a blank was established which did not have the experimental material and IL-1β, and the manifested inhibited amount of the adhesion molecules on HUVEC was obtained by equation. Table 3 shows the results.

[0060]

[Table 2] Adhesion molecules manifested inhibition rate (%) = [1-(experimental materials O.D. value- blank O.D. value) / (control O.D. value -blank O.D. value) x 100 (within the equation, the O.D. value expresses the absorbed light intensity)

[0061]

[Table 3]

Plant Extracts	E-selectin Manifestation Inhibition (%)	ICAM-1 Manifestation Inhibition (%)
Chrysanthemum	16	22
Terminalia chebula Retzus	0	91
Water caltrops	0	31
Japanese-radish grass	1	0
Pomegranate actual skin	62	0
Bearberry leaf	0	53
YARO	28	25
Rose	0	44
Cumin	62	24
Cloves	5	21

From the results of Table 3, it was thought that the inhibition of the cell adhesion was by the pomegranate real skin and the cumin as the principal ingredients which inhibited the manifestation of E-selectin, and that roses were the principal inhibitor of the manifestation of ICAM-1. In addition, it was surmised that the Japanese radish grass

had prevented cell adhesion according to factors other than those mentioned.

[0062] Experiment 3 Leukocyte E-selectin adhesion inhibition test: By the following methods, a leukocyte and the substance which acts antagonistically to leukocytes were examined for E-selectin.

[0063] - Add human recombinant E-selectin (R&D Systems, product) 800ng/ml PBS+ every 100μ l to the production 96 well microplate (Immulon 4, product from Dynatech) of the plate which carried out the coating of the E-selectin, and carry out a coating at 4°C overnight. Block with 250 μl of PBS+ solutions which contains BSA (bovine serum albumin) 1% at room temperature for 1 hour, and then wash with PBS+.

[0064] - Suspend cells for the HL60 (human bone marrow tumor cell) 2 x 10^7 units that were cultivated by the conventional preparation method of fluorescence labeled HL60 cell in BCECF-AM (Molecular Probe, product) using 50μg / 1.5ml of culture media. Incubate at 37°C for 30 minutes, and fluorescence labeling of the cell was carried out. Wash

using the culture liquid washes and prepare 2 x 10^6 units/ml using PBS+ which contains BSA 0.1%.

[0065]

To every well of flat bottom plate that was coated with E-selectin, add (as a control, add 50µl of only 0.1% BSA-PBS+) 50µl of the experimental extract of arbitrary concentration (final concentration 0.4 or 2mg/ml 0.1% BSA-PBS+), and let stand at room temperature for 20 minutes and then carry out a reaction. Next, add 1 x 10^5 units of fluorescent labeled HL60 cells (50µl of the above-mentioned solution of 2 x 10^6 units/ml), and react for 10 minutes at room temperature. A plate washer machine (DIA-Washer (2) Diatron product) is used, and every well is washed twice using 1.0ml of the culture liquid (0.5%BSA-PBS+), removing the non-adhered cells. After suctioning off the washing liquid, 100µl of a 0.1% SDS solution was added, and the solution was let stand for 20minutes at room temperature, and the cells that has adhered to the E-selectin were dissolved. Measurements were made at Ex490nm and Em530nm of the fluorescence intensity of the solution using a fluorescence plate reader (MTP-100F, product from Corona). A cell adhesion inhibition test was performed for a control solution using the wells as blanks

for those wells that were not coated with E-selectin. The rate of cell adhesion inhibition was calculated according to the following equation. Results are shown in Tables 4 and 5.

[0066]

[Equation 3] Cell adhesion inhibition rate (%) = [1-{(experimental F value- blank F value)/ (control F value - blank F value)}] x 100 (in the equation, F value indicates the fluorescence intensity)

[0067]

[Table 4]

Plant Extracts (50% ethanol extract, 0.4mg/ml)	Leukocyte Cell Adhesion Inhibition Rate (%)
Solidago japonica	99
Polygala root	98

[0068]

[Table 5]

Plant Extracts (50% ethanol extract, 2.0 mg/ml)	Leukocyte Cell Adhesion Inhibition Rate (%)
Gymnema	100
Herba Dianthi Superbi	100
Platycodi radixes	100
Pot marigold	91
Comfreys	85
Red pepper	83
SHICHOUTAN	81
Phycho	78
Anemarrhena rhizomes	68
Hop	68
Lonicera japonica	68

As mentioned above, the active ingredient of this invention can inhibit leukocyte cell adhesion.

[0069] Experiment 4 Toxicity experiment for vascular endothelial cells (cell form, cell proliferation ability): formation changes were determined visually by a handstand microscope, and using the Cell Counting Kit (Dojindo Chemistry) which followed the usual methods for determining cell proliferation ability, there was an evaluation by indexing the incorporation of WST-1 (tetrazolium salt) by the human vascular endothelial cell (HUVEC). There was culturing of the human vascular endothelial cells to make them confluent in 96 wells of a level plate (flat bottom plate), and experimental material was added so that the final concentration became 1mg/ml. After letting stand for 24 hours at 37 °C, the culture liquid was removed, and new culture liquid was added, making the volume 100μl/well. Furthermore, there was added 10μl/well of the Cell Counting Kit solution, and after being cultured for 2 hours in a CO_2 incubator, a color reaction was implemented. After the reaction, measurements were made of the absorbance using a microplate reader (measurement wavelength of 450nm, 630nm of control wave length). Blanks were set as not having inserted any experimental material for the human vascular endothelial cells, and using the following equation, a cell proliferation ability inhibition rate was obtained.

This result, as shown in Table 6, indicates a low toxicity for any of these plants for vascular endothelial cells.

[0070]

[Equation 4] Cell proliferation ability inhibition rate (%) = [1 - (experimental material O.D. value - blank O.D. value)/ (control O.D. value- blank O.D. value)] x 100 (in the equation, the O.D. value indicates a value for absorbance intensity)

[0071]

[Table 6]

Plant Extract	Form Change	Cell Proliferation Ability Inhibition Rate (%)
Chrysanthemum	Nothing special	0
Terminalia chebula Retzus	Nothing special	19
Water caltrops	Nothing special	32
Japanese-radish grass	Nothing special	0
Pomegranate actual skin	Nothing special	24
Bearberry leaves	Nothing special	0
YARO	Nothing special	0
Roses	Nothing special	32
Cumin	Nothing special	13

Cloves	Nothing special	41
Solidago japonicas	Nothing special	39
Polygala roots	Nothing special	48
Gymnema	Nothing special	29
Comfreys	Nothing special	0
Herba Dianthi Superbi	Nothing special	-
Platycodi radix	Nothing special	2
Pot marigold	Nothing special	4
Red pepper	Nothing special	3
SHICHOUTAN	Nothing special	30
Phycho	Nothing special	24
Anemarrhena rhizomes	Nothing special	11
Hop	Nothing special	0
Lonicera japonica	Nothing special	17

Working Example 1 Tablets

Each ingredient was used according to the following blending weights, and prepared according to usual methods

[0072]

Ingredient	Blending Weight (%)
Manufacturing Example 3 Chrysanthemum 50% ethanol extract	83.4
Corn starch	4.8
Crystal cellulose	8.3
Carboxymethylcellulose sodium	3.5

Working Example 2 Granule

Each ingredient used the following blending weights, and the granule was prepared according to the conventional method.

[0073]

Ingredient	Blending Weight (%)
Manufacturing Example 2 Chrysanthemum methanol extract	25.3
Lactose	58.9
Corn starch	14.7
Hydroxypropylcellulose	1.1

Working Example 3 Powder

Each ingredient used the following blending weights, and the powder was prepared according to the conventional method.

[0074]

Ingredient	Blending Weight (%)
Manufacturing Example 2 Chrysanthemum methanol extract	10.0
Lactose	80.0
Corn starch	10.0

Working Example 4 Grain Agent

Each ingredient used the following blending weights, and the powder was prepared according to the conventional method.

[0075]

Ingredient	Blending Weight (%)
Manufacturing Example 2 Chrysanthemum methanol extract	0.1
D-manitol	44.7
Lactose	42.0
Crystal cellulose	10.0
[Unknown]	3.2

Working Example 5 Capsule Agent

Each ingredient used the following blending weights, and the capsule was prepared according to the conventional method.

[0076]

Ingredient	Blending Weight (%)
Manufacturing Example 3 Chrysanthemum 50% ethanol extract	57.7
Lactose	26.9
Corn starch	14.6
Magnesium strearate	0.8

Working Example 6 Injection

Each ingredient used the following blending weights, and the injection was prepared according to the conventional method.

[0077]

Ingredient	Blending Weight (%)
Manufacturing Example 1 Chrysanthemum boiled water extract	0.5
Hydrochloric acid	5.0
Sodium hydroxide	Appropriate weight
Distilled water used for injection	Appropriate weight

Working Example 7 Tea agent (powder)

Each ingredient used the following blending weights, and the tea agent was prepared according to the conventional method.

Ingredient	Blending Weight (%)
Manufacturing Example 1 Chrysanthemum boiled water extract	16.5
Dextrin	82.5
Vitamin type	1.0

Working Example 8 Cornflakes

Each ingredient used the following blending weights, and the cornflakes were prepared according to the conventional method.

[0079]

Ingredient	Blending Weight (%)
Manufacturing Example 2 Chrysanthemum methanol	0.2

Chrysanthemum methanol extract	
Corn (?)	87.1
Sugar	11.0
Table salt	1.7

Working Example 9 Instant Noodles

Each ingredient used the following blending weights, and the instant noodles were prepared according to the conventional method.

[0080]

Ingredient	Blending Weight (%)
Manufacturing Example 2 Chrysanthemum methanol extract	0.1
Wheat Flour	79.7

Plant protein	9.7
Starch	5.0
Other	5.5

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(51) Int. Cl.[6]	Classification	FI	
A61K 35/78		A61K 35/78	S
			T
			V
			W
A23L 1/16		A23L 1/16	A
1/30		1/30	B
A61K 31/00	635	A61K 31/00	635B
	643		643D

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